RIBOSOMAL RIBONUCLEIC ACIDS FROM THE CRUSTACEAN *ARTEMIA*

Jesús CRUCES, Jesús SEBASTIAN and Jaime RENART
Instituto de Enzimología y Patología Molecular del CSIC, Facultad de Medicina de la Universidad Autónoma, Arzobispo Morcillo, 4, Madrid-34, Spain

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1. Introduction

Ribosomal RNAs (rRNAs) in eukaryotic organisms comprise 4 well-defined molecular entities, with standard sedimentation coefficients of 28 S, 18 S, 5.8 S and 5 S. 5.8 S rRNA is attached to 28 S rRNA by hydrogen bonding [1]. Since the discovery [2] that some insect 28 S rRNA is dissociated into two 18 S products after different denaturing treatments (heat, urea or dimethylsulfoxide, Me_2SO), a large number of other organisms have been shown to have a hidden break in the 28 S rRNA, most of them being protostomes [3].

We are interested in the control of gene expression during the embryogenesis of the crustacean *Artemia*, particularly of the rRNA genes. The restriction map of these genes has been reported [4] and we now present the molecular species which are present in the '28 S rRNA complex' and the other rRNAs from *Artemia* embryos.

2. Materials and methods

Artemia cysts were obtained from San Francisco Bay Brand Inc. (Metaframe Co., Menlo Park, CA 94025; batches 2018 and 1808). *Artemia* larvae (nauplii) were grown as in [5]. Yeast 5 S and 5.8 S rRNAs were obtained as in [6]. Tobacco mosaic virus (TMV) RNA was obtained from virions by phenol extraction. *Escherichia coli* rRNAs were obtained as below. For extraction of rRNAs from *Artemia* cysts, they were dechorionated as follows: cysts were hydrated overnight at 4°C in at least 50 vol. distilled water and then treated with 5 vol. 5% NaClO at 4°C for 30 min with stirring. Dechorionated cysts were then extensively washed with cold distilled water.

rRNA from nauplii or dechorionated embryos was obtained as follows: embryos were suspended in 2 vol. 20 mM Hepes (pH 7.6), 70 mM KCl, 9 mM $Mg(CH_3COO)_2$, 0.1 mM EDTA, 1 mM DTT, 5% glycerol [7] supplemented with 250 μg heparin/ml and homogenized in a glass/Teflon homogenizer. The extract was centrifuged at 30 000 × *g* for 30 min and then at 105 000 × *g* for 2 h. The ribosomal pellet was rinsed with sterile water and resuspended in 50 mM $NaCH_3COO$ (pH 5.1), 25 mM NaCl, 3 mM $Mg(CH_3COO)_2$ with the aid of 0.05 vol. 0.5 mm sterilized glass balls. SDS was added to 2% final conc. and the mixture extracted with phenol (saturated with the sodium acetate buffer) 3 times. The aqueous phase was made 0.3 M in $NaCH_3COO$ and precipitated with 2 vol. cold ethanol at −20°C overnight. After centrifugation at 12 000 × *g* for 10 min, the rRNA pellet was resuspended in 10 mM Hepes (pH 7.5), 100 mM NaCl, 2 mM EDTA, 0.1% SDS. Alternatively, the aqueous phase was extracted 3 times with ether and used immediately. Large and small rRNAs were separated in 7.5–30% (w/w) sucrose gradients in 10 mM Hepes buffer. rRNA could be loaded at 2.5 mg/gradient in the SW25.2 rotor. Gradients were run at 24 000 rev./min for 21 h at 10°C. (All buffers used were treated with 1% diethylpyrocarbonate and sterilized.)

Gel electrophoresis was done as in [8] with or without the glyoxal/Me_2SO mixture for denaturing or native gels, respectively.

Abbreviations: Me_2SO, dimethylsulfoxide; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-ethanesulfonic acid; DTT, D,L-dithiothreitol; SDS, sodium dodecyl sulfate

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3. Results and discussion

3.1. *Characterization of* Artemia *rRNAs under native conditions*

When *Artemia* rRNAs are centrifuged in non-denaturing conditions on sucrose gradients, they migrate as 25 S, 17 S and 5 S (using *E. coli* rRNAs as markers) as shown in fig.1. Gel electrophoresis in non-denaturing conditions shows the same values either by directly applying the total rRNA preparation (fig.2(2)) or after their separation on sucrose gradients (fig.2(3,4)). Therefore, *Artemia* rRNAs behave as 25 S, 17 S and 5 S as long as non-denaturing conditions are maintained. These results confirm the sedimentation coefficients in [9] for the large *Artemia* rRNAs.

3.2. *Occurrence of a complex structure in the 25 S rRNA species*

When 25 S rRNA is electrophoresed under denaturing conditions in the presence of Me_2SO and glyoxal [8], there is no RNA above the 23 S marker, but two smaller bands appear in the 17 S region (fig.2(8)). The M_r-value of the 2 bands obtained from the 25 S complex are 0.68×10^6 and 0.62×10^6 (*E. coli* rRNAs are considered as 0.55×10^6 and

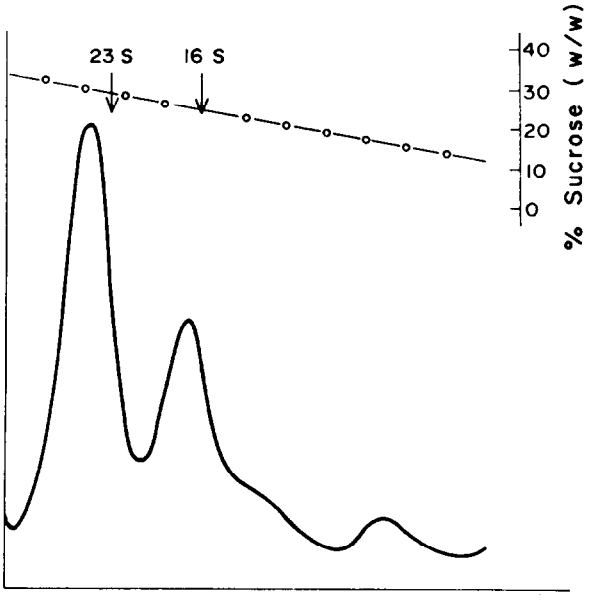

Fig.1. Native sucrose gradient of total rRNA from *Artemia* nauplii. rRNA (100 μg) was centrifuged in a 7.5–30% sucrose gradient in the SW 56 rotor at 50 000 rev./min for 3 h at 10°C, and was fractionated with an Isco gradient-fractionator. Arrows indicate the migration of *E. coli* rRNAs in a parallel gradient.

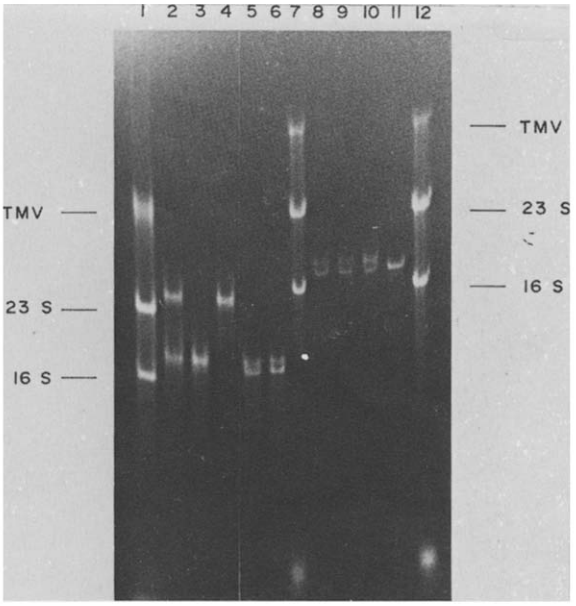

Fig.2. Gel electrophoresis of rRNAs from *Artemia* under different conditions. Vertical slabs (14 × 25 × 0.15 cm) of 1.4% agarose in 10 mM sodium phosphate buffer (pH 7.0) were run at 45 V for 15.5 h as recommended in [8]. Samples were 1–3 μg RNA in the following conditions: native conditions (1) markers, (2) total rRNA, (3) 17 S rRNA, (4) 25 S rRNA, (5) 25 S rRNA incubated 5 min at 60°C, (6) 25 S rRNA incubated 10 min with 75% formamide at room temperature; denaturing conditions (7,12) markers, (8) 25 S rRNA, standard conditions; (9) 25 S rRNA, 17 h at 20°C with glyoxal/Me_2SO, (10) 25 S rRNA, 17 h at 4°C with glyoxal/Me_2SO, (11) 17 S rRNA, standard conditions.

1.03×10^6 for the 16 S and 23 S, respectively [8] and TMV RNA as 2.2×10^6 [10].) A similar effect is shown if the 25 S rRNA is heated at 60°C for 5 min in the absence of glyoxal and Me_2SO (fig.2(5)). On the contrary, 17 S rRNA run under denaturing conditions migrates as one species with M_r 0.63×10^6 (fig.2(11)). Assuming M_r 343 for the sodium salt of a single nucleotide in a polynucleotide chain [11], the size of the rRNAs obtained from *Artemia* embryos are 1980 and 1810 bases for the fragments *a* and *b* of the 25 S rRNA complex, respectively and 1840 bases for the 17 S rRNA.

We have tried other denaturing conditions which do not involve a heating step in order to know whether the 25 S rRNA is of high M_r in vivo (and the 2 fragments are artefactually generated) or it contains a hidden break and the complex is maintained by hydrogen bonding. Incubation with 2% SDS does not dissociate the complex, suggesting that protein is not

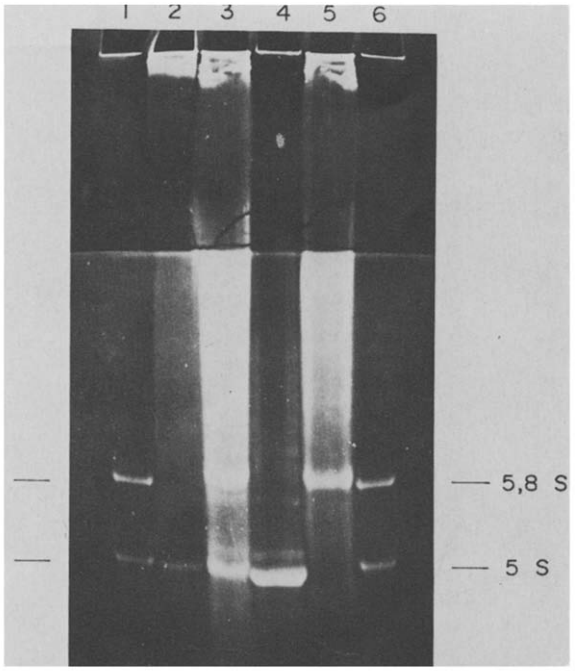

Fig.3. Gel electrophoresis of small rRNAs from *Artemia*. Vertical slabs (14 × 25 × 0.15 cm) were used. The bottom 15 cm were 10% and the upper part 3% polyacrylamide in 10 mM sodium phosphate buffer (pH 7.0). RNA (1–3 μg) were denatured with the standard conditions (8); (1,6) small rRNAs from yeast; (2) 5 S rRNA from *E. coli;* (3) *Artemia* total rRNA; (4) 5 S region from a native sucrose gradient; (5) 25 S region from a native sucrose gradient.

involved (not shown). Incubation with glyoxal and Me_2SO for 17 h at 4°C or at room temperature produces the 2 fragments (fig.2(9) and (10), respectively). The longer incubation at lower temperatures effectively denatures the rRNA giving the same mobility as that obtained after the standard incubation (1 h at 50°C). This experiment shows that the heating step is not responsible for the dissociation. Formamide treatment also induces dissociation. 25 S rRNA treated with 75% formamide for 10 min at room temperature prior to loading to the gel dissociates the rRNA and shows the 2 bands in the 17 S region (fig.2(6)). As the formamide treatment was done only in the sample (no formamide was present in the gel), this result implies that once the 2 chains are dissociated they migrate as independent molecules. The fact that all these treatments, known to abolish hydrogen bonding in RNA, induce the dissociation, strongly supports the idea that the 25 S rRNA is a complex of smaller polynucleotide chains in vivo, probably held together by this type of interaction.

3.3. *Small rRNAs from* Artemia *embryos*

Artemia embryos have 2 small rRNAs in the large ribosomal subunit as do all other eukaryotic organisms. They were identified by running the 25 S and 5 S regions of a native sucrose gradient (see fig.1) on a 10% polyacrylamide gel under denaturing conditions. From the electrophoretic mobilities shown in fig.3, it is concluded that 5.8 S rRNA from *Artemia* is slightly larger than 5.8 S rRNA from yeast, whereas 5 S rRNA is of the same size. The estimated sizes for the *Artemia* small rRNAs are 160 and 121 bases for 5.8 S and 5 S rRNAs, respectively (markers used were: 5.8 S rRNA from yeast, 158 bases; 5 S rRNA from yeast, 121 bases and 5 S rRNA from *E. coli*, 120 bases [12]). The presence of degradation products in the gel shown in fig.3 is likely to be due to the lability of the 25 S rRNA complex.

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